AMENDMENTS TO THE CLAIMS

1-31 (canceled)

32. (currently amended) A method of ~~screening for a mutation in~~ detecting a CGI-69 polynucleotide comprising ~~comparing a nucleic acid sequence to the~~ detecting a polynucleotide comprising a nucleic acid sequence of SEQ ID NOs:1 or 2.

33 34 (canceled)

35. (previously presented)The method of claim 32, wherein the nucleic acid sequence is SEQ ID NO:1.

36. (previously presented)The method of claim 32, wherein the nucleic acid sequence is SEQ ID NO:2.

37. (canceled)

38. (currently amended) The method of claim 32, wherein ~~the screening is by polymerase chain reaction~~ the detecting comprises amplifying the polynucleotide sequence comprising a nucleic acid sequence of SEQ ID NO:1 or 2 by polymerase chain reaction.

39. (currently amended) The method of claim 32, wherein the ~~screening~~ detecting comprises hybridizing a probe to the polynucleotide comprising a nucleic acid sequence of SEQ ID NO:1 or 2 ~~by nucleic acid hybridization~~.

40. (currently amended) The method of claim 32, wherein the polynucleotide comprising a nucleic acid sequence of SEQ ID NO:1 or 2 is detected in a sample ~~is screened~~.

41. (previously presented) The method of claim 40, wherein the sample is from a mammal~~mammalian~~.

42. (previously presented) The method of claim 41, wherein the sample is from a human.

43. (currently amended) The method of claim ~~32~~40, wherein the sample ~~is~~ comprises at least one member selected from the group consisting of blood, serum, cells ~~or~~ and tissue.

44. (new) A method of detecting a CGI-69 polynucleotide, comprising hybridizing under stringent conditions a probe comprising at least a portion of a polynucleotide comprising a nucleic acid sequence of SEQ ID NO:1 or 2 to a nucleic acid sample; and

identifying the CGI-69 polynucleotide in the sample by detecting a hybridization signal.

45. (new) A method of detecting a CGI-69 polynucleotide comprising detecting a polynucleotide encoding a polypeptide comprising an amino acid sequence of SEQ ID NO:3 or 4.

46. (new) The method of claim 45, wherein the encoded polypeptide comprises the amino acid sequence of SEQ ID NO:3.

47. (new) The method of claim 45, wherein the encoded polypeptide comprises the amino acid sequence of SEQ ID NO:4.

48. (new) The method of claim 45, wherein the detecting comprises amplifying the polynucleotide sequence encoding an amino acid sequence of SEQ ID NO:3 or 4 by polymerase chain reaction.

49. (new) The method of claim 45, wherein the detecting comprises hybridizing a probe to the polynucleotide encoding a polypeptide comprising an amino acid sequence of SEQ ID NO:3 or 4.

50. (new) The method of claim 45, wherein the polynucleotide encoding a polypeptide comprising an amino acid sequence of SEQ ID NO:3 or 4 is detected in a sample.

51. (new) The method of claim 50, wherein the sample is from a mammal.

52. (new) The method of claim 51, wherein the sample is from a human.

53. (new) The method of claim 50, wherein the sample comprises at least one member selected from the group consisting of blood, serum, cells and tissue.

REMARKS

Claims 32, 35-53 are active and pending. Support for amended claim 32 can be found on page 8, line 4 and page 8, lines 19-23 and the claims as filed in Paper No. 11. The amendment to claim 43 corrects an improper dependency. The amendments contain no new matter.

Substance of the interview

Applicants' representatives would like to thank Examiners Sakelaris and Myers for the meeting of June 11, 2003, and for their many helpful suggestions and comments.

Representatives for Applicants provided a synopsis of the application, followed by a discussion concerning utility and current Office guidelines. Examiners Sakelaris and Myers suggested that art be used to demonstrate that uncoupling proteins represent a relatively small group of proteins to support the utility; such a references has been provided (Ledesma *et al.*, 2002). Finally, Examiners Sakelaris and Myers suggested amending the claims to read "the detection of SEQ ID X" would advance prosecution. Applicants have amended the claims to read as such.

Case synopsis

Metabolic diseases, such as obesity, reflect an imbalance between appetite/caloric intake and energy expenditure (page 1, lines 16-17). Metabolic diseases represent a serious public health concern worldwide, as obese individuals are susceptible to an array of disorders, including heart disease, high blood pressure, Type II diabetes/insulin resistance, and stroke (page 1, lines 6-11).

The bulk of animal tissue oxygen consumption is driven by a finely balanced system in which the rate of mitochondrial fuel catabolism is regulated by the flow of electrons down the electron transport chain (page 3, lines 6-8). Concomitant pumping of protons outward across the mitochondrial inner membrane establishes the proton electrochemical gradient that is responsible for driving ATP synthesis *via* the inward flow of protons through the F_1F_0 ATP synthase (page 3, lines 8-11). Although fuel combustion, electron transport, proton flux, and ATP turnover are intimately coupled, proton leak across the inner mitochondrial membrane can occur in the

absence of ATP synthesis (page 3, lines 11-14). This phenomenon, uncoupling, reduces the net synthesis of ATP relative to the fuel consumed. Identification of components that contribute to uncoupling or proton leak are useful for treating obesity and other metabolic disorders arising from perturbations in energy balance (page 3, lines 19-21). The Applicants have described such a component, CGI-69.

Using an art-accepted model system to study metabolism, mouse brown adipose tissue (BAT) (*e.g.*, see Nicholls *et al.*, 1984 and Bing *et al.*, 2000), the murine ortholog of CGI-69 was discovered to be up-regulated two-fold in BAT when the mice were subjected to a cold acclimation regime (page 83, line 27 to page 84, line 6). BAT plays a vital role in thermogenesis and maintenance of body weight (page 3, lines 22-26). The structure of CGI-69 encompasses, among other structures, 4 mitochondrial carrier domains, a mitochondrial localization signal, and most remarkably, 3 regions of mitochondrial energy transfer motifs, which are present in known uncoupling proteins (page 84, lines 6-12).

Human orthologs of CGI-69 were then isolated, resulting in the identification of four molecules: (1) wild-type CGI-69 (SEQ ID NOs:2 (polynucleotide) and 4 (polypeptide); Lai *et al.*, 2000; GenBank Accession NM_016016); (2) a novel splice variant, the "long form," CGI-69_L, having an 8 amino acid insert after position 64 and a mutation at position 64 where a leucine is substituted for a tryptophan; (3) CGI-69^{F239L} variant, which has a leucine substituted for a phenylalanine at position 239; and (4) CGI-$69_L{}^{F247L}$, which has a leucine substituted for a phenylalanine at position 247 (page 85, lines 7-13).

Armed with these findings and using RT-PCT, it was then determined that mice testes and BAT express wild-type CGI-69 (page 85, lines 13-15). Human wild-type CGI-69 expression was similar to that of mouse, being observed in the kidney, liver and spleen in humans (page 85, lines 13-15).

To test for CGI-69's ability for uncoupling, a carboxy-FLAG-tagged CGI-69 construct was used and over-expressed in human 293 kidney cells. The mitochondrial membrane potential ($\Delta\psi_m$) in CGI-69-expressing cells was diminished as much as those over-expressing the known uncoupling protein, UCP3, indicating that CGI-69 is an uncoupling protein when fused at the carboxy terminus with FLAG (page 86, lines 11-15).

Thus human CGI-69, which murine equivalent was observed to be two-fold up-regulated in cold-acclimated mice, has a similar role in metabolism in human cells. Human CGI-69

localizes to mitochondria, is expressed in similar tissues as in mice, and can act as an uncoupling protein under appropriate conditions.

REQUEST FOR RECONSIDERATION

Rejection under 35 USC 101, second paragraph

The rejection of the claims under 35 USC 101 is respectively traversed. CGI-69 is an uncoupling protein, a member of a small class of proteins, as indicated by Ledesma *et al.* (2002).

CGI-69 proteins are up-regulated in BAT in cold-acclimated mice, indicating a role in metabolism (page 6, lines 18-25; page 83, lines 27-31). CGI-69 is targeted to the mitochondria, and under appropriate conditions can act as an uncoupling protein (page 6, lines 9-11; page 86, lines 11-12). Uncoupling proteins represent a very small group of proteins, represented by five UCP homologues in mammals (Ledesma *et al.*, 2002). These proteins "are transporters, present in the mitochondrial inner membrane, that mediate a regulated discharge of the proton gradient that is generated by the respiratory chain [. . .] which can function in thermogenesis" (Ledesma *et al.*, 2002; Abstract). Because of the very few members of the uncoupling protein family, one of skill in the art would recognize the utility of a new member of this class. Metabolic disorders, including obesity, affect millions of individuals worldwide, impacting individual health and incurring substantial economic and personal costs: in the United States alone, the Centers for Disease Control and Prevention acknowledge that overweight and obese individuals represent approximately 61% of the adult population.

Because the claimed invention has a credible, specific and substantial utility, withdrawal of this ground of rejection is respectfully requested.

Rejection under 35 USC 112, first paragraph

The rejection of the claims under 35 USC 112 is respectfully traversed. Because the invention has a credible, specific and substantial utility, one of skill in the art will know how to use the invention. Specifically, because CGI-69 is a member of a small class of molecules, the uncoupling proteins (Ledesma *et al.*, 2002), one of skill in the art would recognize its utility and would know how to use the molecule. Withdrawal of this ground of rejection is respectfully requested.

Rejection under 35 USC 112, second paragraph

Rejection of the claims under 35 USC 112, second paragraph, have been obviated by amendment.

References (provided herewith)

Bing, C., M. Brown, P. King, P. Collins, M.J. Tisdale and G. Williams. 2000. Increased gene expression of brown fat uncoupling protein (UCP)1 and skeletal muscle UCP2 and UCP3 in MAC16-induced cancer cachexia. *Cancer Res.* 60:2405-2410.

GenBank Accession NM_016016. 2000. References the Lai *et al.* (2000), below.

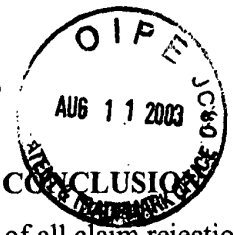
Lai, C.H., C.Y. chou, L.Y. Ch'ang, C.S. Liu and W. Lin. 2000. Identification of novel human genes evolutionarily conserved in *Caenorhabditis elegans* by comparative proteomics. *Genome Res.* 10:703-713.

Ledesma, A., M. Garcia de Lacoba and E. Rial. 2002. The mitochondrial uncoupling proteins. *Genome Biology* 3:2015.1-1015.9.

Yu, X.X., D.A. Lewin, A. Zhong, J. Brush, P.W. Schow, S.W. Sherwood, G. Pan and S.H. Adams. 2001 Overexpression of the human 2-oxoglutarate carrier lowers mitochondrial membrane potential in HEK-293 cells: contrast with the unique cold-induced mitochondrial carrier CGI-69. *Biochem. J.* 353:369-375.

Reference (not provided herewith)

Nicholls, D.G. and R.M. Locke. 1984. Thermogenic mechanisms in brown fat. *Physiol. Rev.* 64:1-64.



CONCLUSION

Reconsideration and withdrawal of all claim rejections is respectfully requested. Applicants believe that all claims in the present application are in condition for allowance.

Should the Examiner have any questions, or would like to discuss any matters in connection with the present application, the Examiner is invited to contact the undersigned at (312) 876-8936.

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